Lecithotrophic development of *Hoplodoris nodulosa* (Angas) (Opisthobranchia, Gastropoda)

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ABSTRACT

The intertidal nudibranch *Hoplodoris nodulosa* from New South Wales produces transparent egg ribbons which contain 400 to 700 ova. The zygotes range from 137 to 141 μm in diameter. Development up to hatching takes 16 days at 22-23°C. Embryos develop in a similar fashion to those of other nudibranchs with lecithotrophic development. Newly hatched veligers either begin metamorphosis immediately or go through a brief crawling-swimming stage for 24 hours before metamorphosis. They do not show any preference for a particular type of substratum on which to settle and metamorphose. The behaviour of the veligers indicates that this species mode of development is demersal rather than pelagic lecithotrophic.

INTRODUCTION

In Australia a number of dorid nudibranchs belonging to the genus *Hoplodoris* Bergh, 1880 are presently undescribed. This genus is estimated to have at least three species in New South Wales and several others elsewhere throughout the Indo-West-Pacific which all look extremely similar externally but vary tremendously in their internal morphology (Rudman, pers. com.). The present paper describes, for the first time, the egg mass and larval development of one of the more common N.S.W. species, *H. nodulosa* (Angas, 1864), and compares its development with those of other opisthobranchs found in the literature. This information is important if the taxonomy, embryology and zoogeography of different species within the genus are to be understood.

MATERIALS AND METHODS

Hoplodoris nodulosa was collected intertidally from Long Reef, N.S.W. during the autumn of 1976, the summer of 1977 and in February, 1979. All of the nudibranchs collected matched the descriptions of specimens recorded by Thompson (1975), Allan

(1947) (as *Doris (Staurodoris) pustulata)* and Angas (1964) (as *Doris nodulosa).* Specimens and whenever possible their respective egg masses were collected and brought back to the laboratory. The adults were kept in aquaria. Egg masses and newly hatched larvae were kept in glass bowls filled with clean, aerated seawater (33-35‰S) that was changed daily. Identification of the developmental pattern was made from embryos produced in the laboratory. Observations were made with a phase contrast microscope. Photographs were taken with a camera and flash unit attached to the microscope.

RESULTS

Egg masses produced by *H. nodulosa* were type A (as defined by Hurst, 1967) and type a^1 (as defined by Fernandez-Ovies, 1981). They were spirally coiled, sturdy transparent ribbons with double-membraned egg capsules unevenly spaced, two or three layers deep, throughout the gelatinous matrix of each ribbon. The capsules encased one orange-brown zygote and often contained bubbles of gas; the appearance of these bubbles was also observed in egg masses collected in the field. The zygotes were $139 \pm 2.1 \mu m$ (n = 10) in diameter and the number of zygotes per egg mass ranged from 400 to 700.

Development up to hatching took 16 days at 22-23°C. The timing of the main stages of development are presented in Table 1. Some of these stages are illustrated in Plate 1 (A to G).

First and second cleavage were equal, holoblastic and meridonal (Plate 1, A). Cleavage beyond the two cell stage was spiral and at third cleavage formation of the first quartet of micromeres was asynchronous. During the blastula stage embryos were flattened dorso-ventrally and developed two transparent anal cells postero-ventrally, 24 hours after gastrulation.

The process of gastrulation was a mixture of epiboly and invagination, resulting in a blastopore groove developing along the mid-ventral axis through the sagittal plane of the body. At this stage, embryos took on a heart-shaped appearance (Plate 1, B). Eventually, the groove closed up except for a small light-coloured blastopore located at the antero-ventral end of the embryo. The anal cells at this time could be seen on the postero-ventral side and to the left of the sagittal plane.

During the trochophore stage, embryos were triangular in shape. At this time they were too opaque for observations on invagination of the stomodoeum or shell gland.

During the early veliger stage (Plate 1, C), embryos developed metapodial and velar rudiments. The marginal cells of the rudimentary velum developed short cilia and the large clear anal cells migrated mid-ventrally to the right side of the body. The shell gland everted and spread across the dorsal and lateral surfaces of the embryo, nearly completing the embryonic shell. During this period the advancement of the secretion of the shell over the antero-dorsal surface of the embryo was marked by a ridge of hyaline cells, which stopped just at the neck of the rudimentary velum. At this time the visceral mass was undifferentiated.

The first signs of movement occurred during the middle veliger stage. Embryos at this time could be seen slowly and smoothly rotating inside their capsules. During this stage a mantle cavity developed and the rudimentary velum became bilobed. The rudimentary metapodium became longer and narrower, and the ventral side or sole developed short cilia like that found on the velum. Towards the end of the middle veliger the shell had completely formed, but it remained attached to the cephalopedal region along the anterior margin of the shell aperture by the mantle. At the beginning of the middle veliger the visceral mass separated from the shell, forming a large perivisceral cavity. Towards the end of this stage the viscera had differentiated into a foregut, midgut or stomach with two asymmetrical digestive diverticula, and a hindgut. In the postero-lateral region of the embryos, and just posterior to the anus, a large clear vesicle developed. This vesicle remained right through metamorphosis and into the juvenile stage. It may have been the secondary larval kidney, since it was retained at the juvenile stage; according to Horikoshi this also happened in *Philine denticulata* (Adams) (cited in Bonar, 1978).

Embryos at the late veliger stage had a well developed cephalopedal region (Plate 1, D). The foot possessed an operculum and the velum (which also had a subvelum) was composed of thick cells with locomotory cilia. The mantle cavity was well developed, especially on the right side, and the mantle was detached from the shell, allowing the embryos to retract completely into the shell. At this stage embryos had developed a pair of small statocysts which were barely distinguishable at the proximal end of the foot. A faint pair of eyes could be seen at the base of the neck of the velum; they became more apparent after hatching. The foregut during the late veliger became fully differentiated. The mouth appeared as a large, deep ciliated pit situated antero-ventrally between the two velar lobes. The oesophagus was straight, short and wide, and entered the stomach anteriorly. The semi-transparent stomach was kidney shaped and positioned between, but slightly posterior to, the midgut diverticula. The left diverticulum was approximately three times as large as the right.It was composed of a number of small cells of which the peripheral ones were empty while most of the inner cells were partially filled with yolk. The right midgut diverticulum was a small, spherical organ that was densely pigmented. The hindgut was a thin, transparent tube that left the posterior end of the stomach dorsally and looped upwards across the dorsum to the right side of the mantle cavity, where it entered the anus. The nephrocysts (or paired 'unicellular primitive kidneys') which were translucent and difficult to discern were situated in the dorso-lateral region of the neck of the velum, adjacent to the statocysts. The clear vesicle increased in size during the late veliger but did not appear to communicate with the exterior. The larval retractor muscle was not noticed during the late veliger but it was detected after hatching in the larvae. The muscle was thin, translucent, fibrous and difficult to see.

Hatching began on the 16th day after oviposition. The newly hatched lecithotrophic veligers had transparent, sinistrally coiled, type 1 shells (as defined by Thompson, 1961). The arrangement of their visceral mass was hyperstrophic. The length of the newly hatched larva along the longitudinal axis was $167 \pm 2.4 \mu m$ ($n = 10$). Veligers were negatively phototactic and they spent most of their time crawling, although they were capable of brief periods (less than 10 seconds) of swimming. The foot rather than the velum was the principal organ of locomotion (Plate 1, E). While swimming some of the veligers would partially expand their velar lobes for a few seconds but they were never expanded, even partially, when crawling. One-third of the veligers started metamorphosis during or immediately after hatching, while the other two-thirds started about 24 hours later. Those that delayed metamorphosis eventually stopped swimming altogether and settled on to the surface of their egg masses, the bottom of their glass bowls or on to algal filaments.

The entire process of metamorphosis lasted about 24 hours, regardless of when it began, and by the second day after hatching all veligers had developed into the juvenile benthic form. The onset of metamorphosis was characterized by four visible events which happened concurrently: firstly, within the mantle cavity, the mantle reflexed over the entire dorsum of the veliger and spread posteriorly; secondly, resorption of the velum commenced; thirdly, the eye spots darkened; and fourthly, the propodium and metapodium thickened distally.

Two hours after settlement the shell and operculum were cast, and a partially developed juvenile could be seen (Plate 1, F). By this time the velum had already been resorbed and two rudimentary cephalic buds had appeared in their place. The statocysts had disappeared and the remains of the larval retractor muscle could be seen as a clump of cells attached to the dorsum mid-posteriorly. The mantle, which had spread half way over the dorsum, could be seen as a thick layer of epidermal cells without any spicules. The darkly pigmented midgut diverticular and stomach, which were anterior to the clear vesicle were located mid-dorsally and had not sunk into the central region of the foot. The renal gland could be seen as a dark spherical mass of cells protruding slightly from the posterior end of the body. The posterior translocation of the renal gland in the early benthic juvenile was the only external evidence that detorsion had occurred during metamorphosis.

In the next 12 hours, the mantle continued to spread over the viscera and began to

extend upwards and laterally to form a mantle skirt, which became embedded with spicules. By the 24th hour after the start of metamorphosis, the viscera had sunk into the central region of the foot. At this stage, the mantle and skirt had expanded over the dorsum but had not enclosed the renal gland; the mantle had also developed papillae reinforced with spicules. Three days after settlement juveniles had two longitudinal rows of dorsal papillae (Plate 1, G). Four of five days after settlement the juveniles had grown two long cephalic projections. Whether these projections were the rhinophoral or oral-tentacle rudiments were not determined. Juveniles at this time still contained yolk reserves and were 570 ± 7 μm (n = 10) in length. They were not reared beyond this stage.

DISCUSSION

According to the morphological and behavioural characteristics of the larva, the development of opisthobranch molluscs can be classified as being planktotrophic (pelagic feeding veliger; Type 1), lecithotrophic (pelagic non-feeding veliger; Type 2), or direct (non-pelagic benthic larva; Type 3) (Thompson, 1967). Direct development can be further divided into capsular metamorphic veliger (metamorphosis occurs within egg capsule; cap. Type 3) or capsular ametamorphic embryogenesis (veliger stage is reduced or omitted; ameta. Type 3) (Bonar, 1978). The larval development of *H. nodulosa* is Type 2. However, the behaviour and settlement requirements of its larvae are different to those of other nudibranchs with Type 2 development. In many respects, the veligers are similar to the demersal larvae described by Mileikovsky (1971) and to a lesser extent a variation of cap. Type 3 larvae.

Unlike the pelagic periods of *Adalaria proxima* (Alder & Hancock) (Thompson, 1958) and *Tritonia hombergi* Cuvier (Thomspon, 1962), the length of time between hatching and completion of metamorphosis for the larvae of *H. nodulosa* can not be divided into distinguishable "obligatory" and "searching" phases for three reasons: first, as in *Cuthona adyarensis* (Rao) (Rao, 1961) and *Cuthona nana* (Alder & Hancock) (Rivest, 1978), the larval period is too short (no more than 48 hours) and during this time the larvae never change their phototactic behaviour. They do not switch from being positively to being negatively phototactic like that of *A. proxima* and *T. hombergi* when changing from the "obligatory" to the "searching" phase (Thompson, 1958 and 1962). Second, *H. nodulosa* veligers do not appear to undergo any obvious external changes like those described for *A. proxima* and *T. hombergi* (Thompson, 1958 and 1962, respectively) or for *Phestilla sibogae* Bergh (Bonar & Hadfield, 1974). Veligers of *H. nodulosa* at hatching already have a well developed or "inflated" propodium for crawling and a mantle which is drawn back some distance from the shell aperture; *P. sibogae* does not show these features at hatching (Bonar & Hadfield, 1974). Third, the larvae of *H. nodulosa* are unable to delay metamorphosis during the "searching" phase as can *A. proxima* and *T. hombergi* (Thompson, 1958 and 1962). Furthermore, unlike these two species and *P. sibogae* (Harris, 1975), they do not require the respective prey species of the adults as a substratum on which to settle. Veligers of *H. nodulsa* metamorphose either on their glass containers or on their egg masses. In this respect, they are similar to those of the aeolids *C. adyarensis* (Rao, 1961) and *C. nana* (Rivest, 1978); the veligers of these species also do not show a preference for any particular substratum on which to settle and metamorphose. The time that metamorphosis begins in *H. nodulosa* varies with each larva but like *Trippa spongiosa* (Kalaart) (Gohar & Soliman, 1967) some begin resorbing their velum immediately after hatching.

The larvae of *H. nodulosa* have a short pelagic phase and no specific settlement requirements. The length of the pelagic period of *H. nodulosa* is somewhere between those of *C. adyarensis* and *C. nana*. Veligers of *C. adyarensis* (Rao, 1961) are pelagic for up to 24 hours while those of *C. nana* are totally non-pelagic and remain on the bottom of their glass containers after hatching, even when beating their vela (Rivest, 1978). Veligers of *H. nodulosa* are capable of short periods of weak swimming for about 24 hours, using primarily their pedal cilia. Furthermore, while swimming they never reached a height of more than 10 cm above the bottom of the aquarium. The feeble swimming technique of the veligers, especially when coupled with their negative phototatic behaviour, suggests that their ability to sustain an existence in the plankton, even for 24 hours, is questionable.

Alternatively, their development could be a variation of demersal development (i.e., larvae spend their pelagic period in the bottom water layers from hatching to the final stages of metamorphosis) (Mileikovsky, 1971).

The development of *H. nodulosa* could also be considered a variation on cap. Type 3 development like that found in *Tenellia pallida* (Alder & Hancock) (Eyster, 1979). Embryos of this species pass through a veliger stage and metamorphose inside their capsules before hatching as benthic juveniles. However, the larvae of *T. pallida* sometime hatch as shelled juveniles capable of swimming, although they do so with great difficulty and never for more than an hour (Eyster, 1979). When this happens the developmental pattern of *T. pallida* is comparable to that of *H. nodulosa* from an ecological perspective. That is, both have a non-pelagic, or at best a brief pelagic period before the completion of metamorphosis.

Detailed investigations on metamorphosis in opisthobranchs have been published by Thompson (1958, 1962), Smith (1967), Tardy (1970), and Bonar & Hadfield (1974). No similar analysis of metamorphosis will be attempted here but certain preliminary observations on some of the morphogenetic events will be considered. However, they need further confirmation by histological sectioning of the larvae.

Until the study of *P. sibogae* (Bonar & Hadfeild, 1974), *Tenellia ventilabrum* Dalyell (Tardy, 1970) and *Elysia chlorotica* (Gould) (Bonar, 1978) the dorsal epidermis in nudibranchs and shell-less sacoglossans was believed to always be derived from the thickened tissue of the mantle prior to, or during, metamorphosis. Evidence of this came from work on *A. proxima* (Thompson, 1958) and *T. hombergi* (Thompson, 1962) and *Aeolidiella alderi* (Cocks) (Tardy, 1970). In these species the thickened tissue of the mantle, within the shelled veliger, everts itself over the dorsal and lateral surfaces of the body. While spreading posteriorly, the everted mantle fuses with the underlying perivisceral membrane (Thompson, 1958, 1962 and Tardy, 1970). However, in *P. sibogae*, it is the lateral surfaces of the foot whose cells proliferate and spread over the visceral mass to form the dorsal integument of the benthic juvenile. Sections through metamorphosing larvae of *E. chlorotica* show the tissue of the mantle and the tissue of the floor of the mantle cavity to be thin, like those of *P. sibogae* (Bonar, 1978). According to Bonar (1978) this evidence along with similar histological evidence for *T. ventilabrum* (Tardy, 1970), suggests that in these two species the dorsal epidermis also originates from the pedal epidermis. The dorsal epidermis of benthic juveniles of *H. nodulosa* appears to originate from the mantle in the manner described for *A. proxima*, *T. hombergi* and *A. alderi*. The observed origin of the dorsal epidermis of *H. nodulosa* and the fact that it has a type 1 protoconch (Thompson, 1961) indicate that *H. nodulosa* is also a representative of Tardy's (1970) type 1 development.

As with most nudibranchs, there is no evidence of 180° twisting of the visceral mass in *H. nodulosa* veligers. The only sign of torsion in *H. nodulosa* is in the migration of the anal cells (at the end of gastrula) from a postero-ventral position to the right side, near the anus. Otherwise, the visceral organs develop in a post-torsional position. During metamorphosis the intestinal loop and the anal complex in the mantle cavity appear to be posteriorly translocated by the spreading of the reflexed mantle. The process of detorsion in *H. nodulosa* appears to be like that described for *A. proxima* and *T. hombergi* (Thompson, 1958 and 1962, respectively).

The short dispersal phase (48 hours at most) and the weak swimming ability of the Type 2 veligers of *H. nodulosa* suggest that this species is unable to cross large expanses of water. This may partly explain why its recorded distribution includes only Australia (Burn, cited by Thompson, 1975). Unless the adults (and their egg masses) can cross large expanses of water by living on floating debris or on the hulls of ships, like that of *Phidiana lynceus* Bergh (Edmunds, 1977), for example, it is unlikely that this species can be widely spread throughout the Pacific. However, according to Kay & Young (1969) it is possible that *Carminodoris nodulosa* (Angas) from Hawaii and *H. nodulosa* from New South Wales are synonymous. An illustration of *C. nodulosa* by Bertsch & Johnson (1981) shows this

nudibranch to be remarkably similar to *H. nodulosa* from eastern Australia. It would be be interesting to compare the larval development of both nudibranchs and to review their taxonomy. Such a comparison would help clarify the geographical range of *H. nodulosa*.

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Table 1. *Hoplodoris nodulosa*. Chronology of developmental stages from oviposition to hatching for embryos incubated at 22-23°C.

Time	**Developmental Stage**
(hours)	
0	Oviposition
4	Explusion of 2nd Polar Body
6	First Cleavage
12	Second Cleavage
13	Third Cleavage
18	Fourth Cleavage
21	Morula
24	Blastula
(days)	
2.8	Gastrula
3.5	Trochophore
4	Early Veliger
6.6	Middle Veliger
10	Late Veliger
16	Hatching

Key to Lettering

Anus (a); anal cells (ac); clear vesicle (cv); eye spot (es); hindgut (hg); larval retractor muscle (lm); left midgut diverticulum (ld); locomotory cilia (lc); mantle (m); metapodium (mp); mouth (m); operculum (op); papillae (p); partially resorbed velum (pv); propodium (pp); renal gland (rg); right midgut diverticulum (rd); rudimentary cephalic projections (cp); rudimentary metapodium (rm); rudimentary velum (rv); shell (sh); shell gland (sg); spicules (sp); statocyst (s); stomach (st); subvelum (sv); velum (v); visceral mass (vm)

Plate 1. *Hoplodoris nodulosa* A. Unfertilized ovum and four-cell stage 12 hr after oviposition. B. Lateral view of 2.8-day old gastrula; note heart-shaped appearance and anal cells. C. Right view of 4-day old early veliger showing anal cells, rudimentary velum and metapodium. D. Ventral view of a fully developed late veliger embryo which is 10 days old. E. Right-lateral view of a newly hatched free-swimming veliger. F. Right-lateral view of a metamorphosing veliger with shell recently discarded; note remains of larval retractor muscle seen as a clump of clear cells. G. Right-lateral view of three-day old juvenile.